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Infectious Morbidity in Long-Term Survivors of Allogeneic Marrow Transplantation Is Associated With Low CD4 T Cell Counts

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Survivors of allogeneic marrow transplants are immunodeficient for at least 1 year after grafting. Multiple defects of immunity have been found; however, it is not known which defect primarily accounts for the high infectious morbidity of these patients. Twenty-nine allograft recipients who were in complete remission of the original disease were examined for the following parameters of immunity at 1 year after transplant: infection score (gauging the number and severity of infections within the 6 months prior to the annual exam), serum total IgM, IgG, and IgA, anti-Haemophilus influenzae IgG, anti-Streptococcus pneumoniae IgG, skin test reactivity, and the blood counts of B cells, $CD4^+$ T cells, $CD8^+$ T cells, and their subsets. THe only parameter inversely correlated with the infection score was $CD4^+$ T cell count ($P = 0.005$ in univariable analysis, $P = 0.06$ in multivariable analysis). We conclude that infectious morbidity of long-term transplant survivors is related to the reconstitution of $CD4^+$ T cells. Am. J. Hematology 54:131–138, 1997

Key words: infectious morbidity; transplant; $CD4^+$ T cells

INTRODUCTION

Allogeneic bone marrow transplantation is a recognized treatment for certain hematologic malignancies, aplastic anemia, and inborn errors of cells originating from the hematopoietic stem cell [1,2]. Immunodeficiency, manifested as increased propensity to develop infections, follows transplantation and lasts for at least 1 year [3–5]. In an attempt to understand the mechanism of the immunodeficiency of long-term (>6 months) transplant survivors, various aspects of their immunity have been assessed over the last 20 years. These studies revealed multiple defects including atrophy of immune organs [6,7], functional asplenia in patients with chronic graft-versus-host disease (GVHD) [8,9], quantitative deficiency of CD4 T cells [10–12], phenotypic abnormalities of CD4 T, CD8 T, and B cells [13–18], functional defects of T and B cells in vitro [19–22], abnormal skin test reactivity [23], abnormal in vivo antibody responses to antigens [24–27], low serum IgG_2, IgG_4, and IgA [28,29], and restricted repertoire of B cells as well as antibodies [30,31]. It is not known which of these abnormalities primarily contributes to the increased susceptibility of long-term survivors to infections. We evaluated several parameters of immunity for correlation with infectious morbidity in order to find the parameter(s) that is (are) related to the susceptibility to infections.

MATERIALS AND METHODS

Patients

Thirty-four allogeneic marrow transplant recipients returning to our Center for their 1-year-posttransplant exam between August 1993 and June 1994 were evaluated by one investigator (J.S.). Relapse of original malignancy developed in 5 of the 34 patients by 2 years after transplant; these patients were excluded from analysis since the recurrent malignancy might have influenced immune function. The clinical characteristics of the remaining 29 patients are given in Table I.

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TABLE I. Clinical Characteristics of the 29 Patients Analyzed

Patient age (years)	35, [29–47], (13–59)[a]
Day (after transplant) of the 1-year-posttransplant exam	370, [361–396], (336–537)[a]
Patient sex	
Males/females	18/11
Donors	
HLA-identical siblings/donors with a greater degree of histoincompatibility[b]	15/14
Pre-transplant diagnosis	
Hematologic malignancy/aplastic anemia	27/2
Pre-transplant diagnosis	
$- \rightarrow -/+ \rightarrow -/- \rightarrow +$ or $+ \rightarrow +$	7/5/17
History of splenectomy	
Yes/no	1/28
Acute GVDH	
Grade 2–4/grade 0–1	20/8[c]
Clinical chronic GVHD at any time before or at the 1-year-posttransplant exam	
Yes/no	22/7
Clinical chronic GVHD at the time of the 1-year-posttransplant exam	
Yes/no	18/11
Treated/untreated with prophylactic antibiotics for more than 90 days within the 6 months prior to the 1-year-posttransplant exam[d]	13/16
Treated/untreated with immune suppressants within 3 months prior to the 1-year-posttransplant exam[e]	14/15
Treated/untreated with IVIG within 6 months prior to the 1-year-posttransplant exam[f]	9/20

[a]Median [25th–75th percentile] (range).
[b]HLA-A, B, and DR-matched unrelated donors (8), HLA-A, B, and DR-matched unrelated parent (1), HLA-A, B, or DR-mismatched donors (5).
[c]Acute GVHD grade was not evaluable in one patient.
[d]Usually treatment was trimethoprim/sulphamethoxazole 160/800 mg p.o. twice a day on Saturdays and Sundays and Penicillin-V 500 mg p.o. twice a day every day.
[e]Usually treated with prednisone (0.5–1.0 mg/kg every other day) plus cyclosporine (approximately 6 mg/kg every other day per os).
[f]Intravenous immunoglobulin (usually 500 mg/kg monthly). Patients were counted as ''treated'' if they received at least 2 doses within the 6 months preceding the 1-year-posttransplant exam.

Patients with hematologic malignancies were conditioned with cyclophosphamide (120 mg/kg) plus either fractionated total body irradiation (12 Gy) or busulfan (16 mg/kg) [32,33]. Patients with aplastic anemia were conditioned with cyclophosphamide (200 mg/kg) and/or anti-T cell antibodies [34]. T-cell depletion of bone marrow was not employed. All 29 patients were given GVHD prophylaxis with methotrexate (day 1, 3, 6, and 11) and cyclosporine daily during the first 6 months after transplant [35]. Grade $\geqslant$2 acute GVHD was treated with high-dose corticosteroids [36]. Four patients were given a short course of anti-T cell antibodies as additional prophylaxis or treatment of acute GVHD: anti-CD5-ricin conjugate (two patients) [37], anti-CD3 (one patient) [38], or anti-CD25 (one patient) [39]. Clinical extensive chronic GVHD was treated with cyclosporine and prednisone [40].

At the time of the 1-year-posttransplant exam, none of the 29 patients had severe neutropenia while two patients had mild neutropenia (0.9 and 1.6×10^9/l). Bone marrow aspirates were normal except for mild hypocellularity in 2 cases, mild hypercellularity in 1 case and inadequate sample in 1 case. Blood or marrow nucleated cells were of donor origin in 28 patients (by Y-chromosome in situ hybridization [41] or variable nucleotide tandem repeat [42]); chimerism was not evaluated in 1 patient.

All patients signed a consent form approved by the Institutional Review Board.

Scoring of Infections

Details on infections developing within 180 days prior to the 1-year-posttransplant exam were obtained by directly questioning the patients, reviewing questionnaires filled in by the patients' primary physicians, and reviewing records that were mailed to use from the primary physicians. Each infection was given a score according to its severity (Table II). Total Infection Score was calculated as the sum of scores of all infections within the 180 days preceding the 1-year-posttransplant exam.

Enumeration of Lymphocyte Subsets

The percent of B cells, T cells, and their subsets was determined by 3-color flow cytometry as published [15,16]. Briefly, B cells were defined as mononuclear

TABLE II. Scoring of Infections

Infection[a]	Score (points)
Severe, managed by a physician in a hospital	5
Moderate, managed by a physician in an outpatient setting	3
Mild, managed by the patient himself/herself[b]	1

[a]An infectious episode lasting 3–4 weeks was scored twice, 5–6 weeks was scored thrice, etc. Skin warts were not counted.
[b]Diarrhea and upper respiratory symptoms of presumed infectious etiology were counted as an infection only if they lasted more than 2 days.

cells (by forward × side scatter characteristics) that expressed CD19 and/or CD20 and did not express CD3, CD14, or CD16. $CD4^+$ T cells were defined as mononuclear cells that expressed CD3 and CD4. $CD8^+$ T cells were defined as mononuclear cells that expressed CD3 and CD8. Absolute blood count of a lymphocyte subset (Abs_{subset}) was calculated, using clinical laboratory leukocyte count + differential, as

$$Abs_{subset} = (Abs_{Ly} + Abs_{Mo}) \times \%_{subset}/100,$$

where Abs_{Ly} = absolute lymphocyte count, Abs_{Mo} = absolute monocyte count, and $\%_{subset}$ = percent subset cells among all mononuclear cells.

Skin Testing

Standard Candida, mumps, and trichophytin solutions, 0.1 ml each, were injected intradermally and read 48 h later. Consequently, 0–4 mm induration was read as negative, 5–9 mm as weakly positive, and >9 mm as positive. The patient was considered reactive if at least one of the three tests was positive, weakly reactive if no test was positive and at least one was weakly positive, and non-reactive if all tests were negative.

Serum Ig Determination

Total IgM, IgG, and IgA levels were measured by standard rate nephelometry. Levels of specific antibodies against Haemophilus influenzae capsular polysaccharide and against pneumococcal capsular polysaccharides (a mixture of 23 common serotypes) were determined by ELISA (Bindazyme™, Binding Site, Birmingham, UK). To increase the specificity of the pneumococcal ELISA for capsular polysaccharides, cell wall polysaccharide was first added to sample dilutions to adsorb non-specific antibodies [43]. Patients were not vaccinated against Haemophilus influenzae or Streptococcus pneumoniae prior to the 1-year-posttransplant exam.

Statistics

Univariable analyses of the association between total infection score and clinical characteristics (listed in Table III) were performed using the Mann-Whitney test (or the Kruskal-Wallis test if more than 2 groups were being compared) for the patient characteristics that were categorized; Spearman's rank-correlation statistic was used for characteristics that had a natural ordering. Univariable analyses of the association between the total infection score and each of the immune parameters measured (listed in Table IV) were performed using Spearman's rank-correlation statistic.

Multivariable analyses were performed using linear regression models. The models were constructed by first building a base model from the clinical characteristics (listed in Table III). Once the base model was constructed, each immune parameter (of those listed in Table IV) was added to the base model in order to assess the contribution of the respective parameter to the total infection score above and beyond the contribution already made by the variables in the base model. The base model and the base model plus immune parameter were compared by the F-test for comparing nested models.

All reported P values are two-sided. No adjustments for multiple comparisons were made in calculating reported P values.

RESULTS

Infections Occurring Within the 180 Days Preceding the 1-Year-Posttransplant Exam

Within the 180 days preceding the follow-up exam, a total of 31 mild, 53 moderate, and 14 severe infections occurred in the 29 patients. The infections are displayed in Table V.

In univariable analysis, the only clinical characteristic that was significantly associated with the total infection score was chronic GVHD, $P = 0.02$ (Table III). The only immune parameter that was significantly associated with the total infection score was the $CD4^+$ T cell count, $P = 0.005$ (Table IV and Fig. 1). Serum IgM, IgG, and IgA levels, skin test reactivity, B cell count, and $CD8^+$ T cell count did not appear significantly associated with the total infection score.

To determine whether the $CD4^+$ T cell count was independently associated with the total infection score, a multivariable analysis was performed using linear regression. The following variables were included into the base model: the presence or absence of clinical chronic GVHD at any time before or at the 1-year-posttransplant exam (cgvhd) and days after transplant (dayspost). Other variables listed in Table III were not included because none of them statistically significantly improved the model. The base model therefore looked like the following:

$$\text{Total infection score} = a + b_1 \times \text{cgvhd} + b_2 \times \text{dayspost}$$

TABLE III. Univariable Analyses Looking for Possible Correlation Between the Total Infection Score and Clinical Characteristics

	Spearman correlation coefficient (ρ) or median total infection score	*P*
Patient age	ρ = 0.26	0.16
Day (after transplant) of the 1-year-posttransplant exam	ρ = −0.21	0.27
Patient sex	Males, 7.5; females, 6.0	0.48
Donor type	HLA-identical sibs, 8.0; donors with a greater degree of histoincompatibility, 5.0	0.36
Pre-transplant CMV serostatus (donor → host)	− → −, 2.0; + → −, 4.0; − → + or + → +, 8.0	0.09
Acute GVHD grade (0–4)	ρ = −0.06	0.75
Clinical chronic GVHD at any time before or at the time of the 1-year-posttransplant exam	Yes, 9.0; no, 3.0	0.02
Treatment with any immune suppressant(s) within 3 months prior to the 1-year-posttransplant exam	Yes, 7.5; no, 5.0	0.80
Treatment with IVIG within 6 months prior to the 1-year-posttransplant exam	Yes, 10.0; no, 5.0	0.08
Treatment with antibiotics for more than 90 days within the 6 months prior to the 1-year-posttransplant exam	Yes, 8.0; no, 5.0	0.44

The multiple correlation coefficient in this model had a value of $R^2 = 0.23$. This means that roughly 23% of the variation in the observed total infection score can be explained by the variables in the base model. $CD4^+$ T cell count (CD4) plus the square of the $CD4^+$ T cell count ($CD4^2$) were then added to the base model:

$$\text{Total infection score} = a + b_1 \times \text{cgvhd} + b_2 \times \text{dayspost} + b_3 \times \text{CD4} + b_4 \times \text{CD4}^2$$

The multiple correlation coefficient in this model had a value of $R^2 = 0.39$. This means that roughly 39% of the variation in the observed total infection score can be explained by the variables in the final model. The addition of the $CD4^+$ T cell count appeared to improve the base model ($P = 0.06$, F-test).

The inclusion of any of the remaining immune parameters (listed in Table IV) to the base model had no significant effect on the improvement of the base model ($P > 0.20$).

Which $CD4^+$ T Cell Subpopulation(s) May Be Related to the Infections?

We were interested whether infectious morbidity was related to the quantity of naive, memory, or both $CD4^+$ T cell subsets. Using CD45RA antigen to distinguish the two subsets [44], the total infection score inversely correlated with both naive and memory $CD4^+$ T cell counts ($P = 0.006$ and $P = 0.01$, respectively, in univariable analyses). Using L-selectin to distinguish the two subsets [45], the total infection score inversely correlated with naive $CD4^+$ T cell count ($P = 0.002$ in univariable analysis) but not with memory $CD4^+$ T cell count.

We also assessed whether infections were related to the quantity of $CD28^+$ or $CD28^-$ $CD4^+$ T cell subsets. The total infection score inversely correlated with $CD28^+$ $CD4^+$ T cell count ($P = 0.004$ in univariable analysis). In contrast, there was a trend towards positive correlation between the total infection score and the $CD28^-$ $CD4^+$ T cell count ($P = 0.10$ in univariable analysis).

In multivariable analyses, similar trends were observed. However, the addition of any of the $CD4^+$ T cell subsets to the linear regression base model did not improve the base model significantly (Table IV).

Infections Occurring Within 1 Year After the 1-Year-Posttransplant Exam

Reliable information on deaths and hospitalizations occurring within 1 year after the follow-up exam was available on all 29 patients. During this interval, three patients died and another three patients were hospitalized (each patient once). The causes of the 3 deaths were CMV pancreatitis and pneumonia, pneumonia of presumed bacterial etiology, and disseminated zoster. The causes of the 3 hospitalizations were respiratory syncytial virus pneumonia, pneumonia of presumed bacterial etiology, and Haemophilus influenzae sinusitis. The remaining 23 patients survived without hospitalization. Because reliable information on infections that did not require hospitalization was not available, a simplified infection score was used: The patients surviving without hospitalization were given a score of 0, those that survived but needed hospitalization were given a score of 1, and those who died were given a score of 2.

By the Spearman rank correlation test, the only clinical characteristics that appeared to be associated with hos-

TABLE IV. Univariable and Multivariable Analyses Looking for Possible Correlation Between the Total Infection Score and the Immune Parameters Measured at 1-Year Posttransplant

		Univariable analyses		Multivariable analyses*	
	n	Spearman correlation coefficient	*P*	R^2	*P*
Serum total IgM	29	+0.07	0.73	—	—**
Serum total IgA	29	−0.08	0.68	—	—
Serum total IgG	29	−0.18	0.33	—	—
Serum total IgG, discounting patients who received IVIG within 2 months prior to the blood draw	23	−0.21	0.33	—	—
Serum Haemophilus IgG	29	−0.15	0.41	—	—
Serum Haemophilus IgG, discounting patients who received IVIG within 2 months prior to the blood draw	23	−0.29	0.18	—	—
Serum pneumococcal IgG	29	−0.05	0.78		
Serum pneumococcal IgG, discounting patients who received IVIG within 2 months prior to the blood draw	23	−0.22	0.31	—	—
Skin test reactivity to recall antigens (non-reactive [0] vs. weakly reactive [1] vs. reactive [2])	23	+0.15	0.50	—	—
B cells[a]	29	−0.20	0.30	—	—
$mIgD^+$ B cells[b]	28	−0.20	0.32	—	—
$mIgD^-$ B cells[c]	28	−0.20	0.30	—	—
$CD4^+$ T cells[a]	29	−0.51	0.005	0.39	0.06
$CD45RA^{high}$ $CD4^+$ T cells[b]	25	−0.54	0.006	0.36	0.11
$CD45RA^{low/-}$ $CD4^+$ T cells[c]	25	−0.49	0.01	0.26	0.20
L-$selectin^+$ $CD4^+$ T cells[b]	28	−0.56	0.002	0.29	0.13
L-$selectin^-$ $CD4^+$ T cells[c]	28	−0.11	0.59	0.22	0.70
$CD28^+$ $CD4^+$ T cells	28	−0.53	0.004	0.32	0.19
$CD28^-$ $CD4^+$ T cells	28	+0.32	0.10	0.37	0.09
$CD8^+$ T cells[a]	29	+0.07	0.74	—	—
$CD45RA^{high}$ $CD8^+$ T cells	25	−0.15	0.47	—	—
$CD45RA^{low/-}$ $CD8^+$ T cells	25	+0.15	0.46	—	—
L-$selectin^+$ $CD8^+$ T cells	26	−0.14	0.48	—	—
L $selectin^-$ $CD8^+$ T cells	26	+0.11	0.59	—	—
$CD28^+$ $CD8^+$ T cells	28	−0.16	0.40	—	—
$CD28^-$ $CD8^+$ T cells	28	+0.16	0.41	—	—

[a]Absolute blood counts of B cells and T cells and their subsets were used.
[b]This subset is rich in naive cells.
[c]This subset is rich in memory cells.
*R^2 is an index of how much variability in the linear regression model can be attributed to the variables included into the model. The *P* value gives the probability that the model with both the base model variables (chronic GVHD and days after transplant) and the immune parameter is better than the model with the base model variables only. R^2 of the base model was 0.23.
**Not significant ($P > 0.20$).

pitalizations/deaths after the 1-year-posttransplant exam were the original total infection score of the 180 days preceding the exam ($\rho = 0.48$, $P = 0.009$) and the presence of chronic GVHD at 1 year after transplant ($\rho = 0.40$, $P = 0.03$). None of the immune parameters measured appeared to be associated with hospitalizations/deaths after the 1-year-posttransplant exam, except for a trend in case of $CD45RA^{high}$ $CD4^+$ T cell count ($\rho = -0.35$, $P = 0.09$), L-$selectin^+$ $CD4^+$ T cell count ($\rho = -0.30$, $P = 0.12$) and $CD28^+$ $CD4^+$ T cell count ($\rho = -0.33$, $P = 0.09$). Due to the low number of hospitalizations and deaths, multivariable analyses were not performed.

DISCUSSION

Here it is reported for the first time that low $CD4^+$ T cell counts in long-term allo-transplant survivors are as-

TABLE V. Infections Occurring Within 180 Days Preceding the 1-Year-Posttransplant Exam

Infections	Number
Respiratory tract infections including sinusitis (etiologic agent was known only in 4 cases: influenza A virus, parainfluenza virus type 1, Staphylococcus aureus, Moraxella catarrhalis)	51
Oral infections (presumed or documented herpes simplex in 6 cases, thrush in 7 cases)	13
Enteritis/colitis (Clostridium difficile in 5 cases, etiologic agent unknown in the remaining 4 cases)	9
Genital infections (presumed or documented herpes simples in 5 cases, vaginitis without a known etiologic agent in 4 cases)	9
Skin infections (presumed viral etiology [zoster] in 1 case, presumed bacterial etiology in 3 cases and presumed fungal etiology in 2 cases)	6
Bacteremia/fungemia/sepsis-like illness (Klebsiella pneumoniae in 1 case, Candida parapsilosis in 1 case, no etiologic agent in 3 cases)	5
Pneumonia (Pneumocystis carinii in 1 case, no etiologic agent in 1 case)	2
Urinary infection, etiologic agent unknown	1
Pericarditis and hepatitis of presumed viral etiology	1
Eye infection, not otherwise specified	1

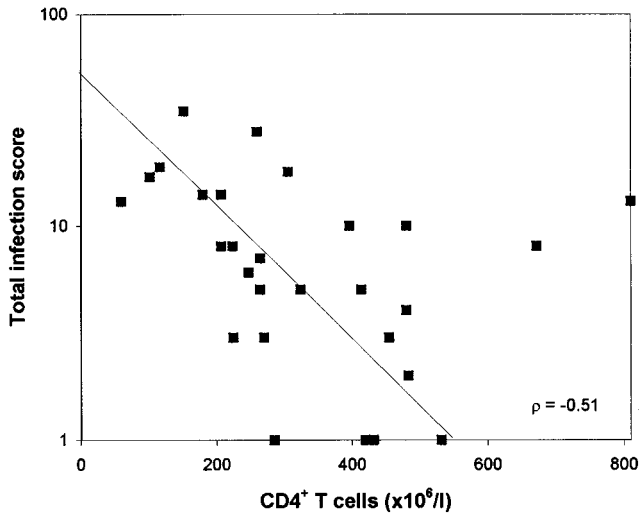

Fig. 1. The association between the total infection score (which takes into account the frequency and severity of infections that occurred within 180 days preceding the 1-year-posttransplant exam) and the absolute $CD4^+$ T cell count. The correlation coefficient (ρ) was calculated by the Spearman rank-correlation method. In this graph with log-scale vertical axis, zero values were arbitrarily assigned the value of 1.

sociated with infectious morbidity ($P = 0.005$). This association appears to be independent of the variables known to be associated with infectious morbidity, i.e., time posttransplant [5] and chronic GVHD [3] ($P = 0.06$).

It cannot be concluded that immune parameters other than $CD4^+$ T cell count (e.g., B cell count or Ig levels) are not associated with infectious morbidity. The power of our study was too small to draw such a conclusion. Also, B cell counts and total serum IgG levels are no longer low in most patients at 1 year after transplant [16,17,29]. It is possible that a correlation of B cell counts or IgG levels with infections would be found if the immune parameters were measured at an earlier time after grafting. Sheridan [46] found a significant association between the incidence of pneumococcal infections at >6 months after transplant with serum IgG_2 levels measured at 3–4 months after transplant.

The trend towards the opposite association of $CD28^+$ and $CD28^-$ $CD4^+$ T cells with infections (Table IV) may shed some light on the function of each of these subsets in vivo. In vitro, $CD28^+$ T cells react to mitogens and antigens whereas $CD28^-$ T cells appear anergic and may suppress the function of the reactive T cells [47–49], hence the common perception of $CD28^+$ cells as reactive and $CD28^-$ cells as anergic or suppressive. However, these observations were made using predominantly $CD8^+$ T cells. Little is known about the scarce $CD28^-$ $CD4^+$ T cells (~2% of $CD4^+$ T cells in normal adults, ~10% of $CD4^+$ T cells in adult marrow transplant recipients at 1 year after grafting) [15]. $CD28^-$ $CD4^+$ T cells do not proliferate upon stimulation with most allogeneic cells [50]; they also suppress the proliferative response of autologous mononuclear cells to allogeneic cells [51]. In contrast, $CD28^+$ $CD4^+$ T cells proliferate upon stimulation with most allogeneic cells [50]; they also possess a contrasuppressor activity [52,53]. The trend towards the positive correlation of $CD28^-$ $CD4^+$ T cells vs. the negative correlation of $CD28^+$ $CD4^+$ T cells with infections in our patients supports that $CD28^-$ $CD4^+$ T cells may be anergic and/or suppressive, and $CD28^+$ $CD4^+$ T cells may be reactive and/or contrasuppressive.

Because of some striking similarities between immunity after transplant and in early life (reviewed in [54–56]), the immunity of long-term transplant recipients has been viewed as analogous to the immunity of normal neonates and infants. However, the association of infectious morbidity with $CD4^+$ T cell count recalls analogy to AIDS patients [57,58] rather than to normal neonates or infants. In contrast to transplant recipients and AIDS patients, normal neonates and infants have high $CD4^+$ T cell counts [15]. Also, whereas normal infants frequently develop infections due to encapsulated bacteria and respiratory viruses and not due to opportunistic pathogens like CMV or fungi [59], AIDS patients and long-term transplant survivors not receiving antimicrobial chemoprophylaxis frequently develop infections due to both encapsulated bacteria and respiratory viruses as well as opportunistic pathogens like CMV and fungi [3,60].

Given the low infectious morbidity/mortality after the 1-year-posttransplant exam and the small number of patients studied, we cannot conclude whether the total

$CD4^+$ T cell count or a $CD4^+$ T cell subset count at 1 year after transplant has a predictive value for developing infections between 1 and 2 years after transplant. Nevertheless, two clinical parameters appeared predictive: the infectious morbidity before the 1-year-posttransplant exam and the presence of chronic GVHD at the time of the exam.

In summary, in long-term transplant survivors the propensity to develop infections appears related at least to the following factors: time after transplant [5], chronic GVHD [3], and $CD4^+$ T cell reconstitution.

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